

Pseudomonas aeruginosa Short-Range Signaling Protein Influence On Biofilm Phenotypic Expression

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Background

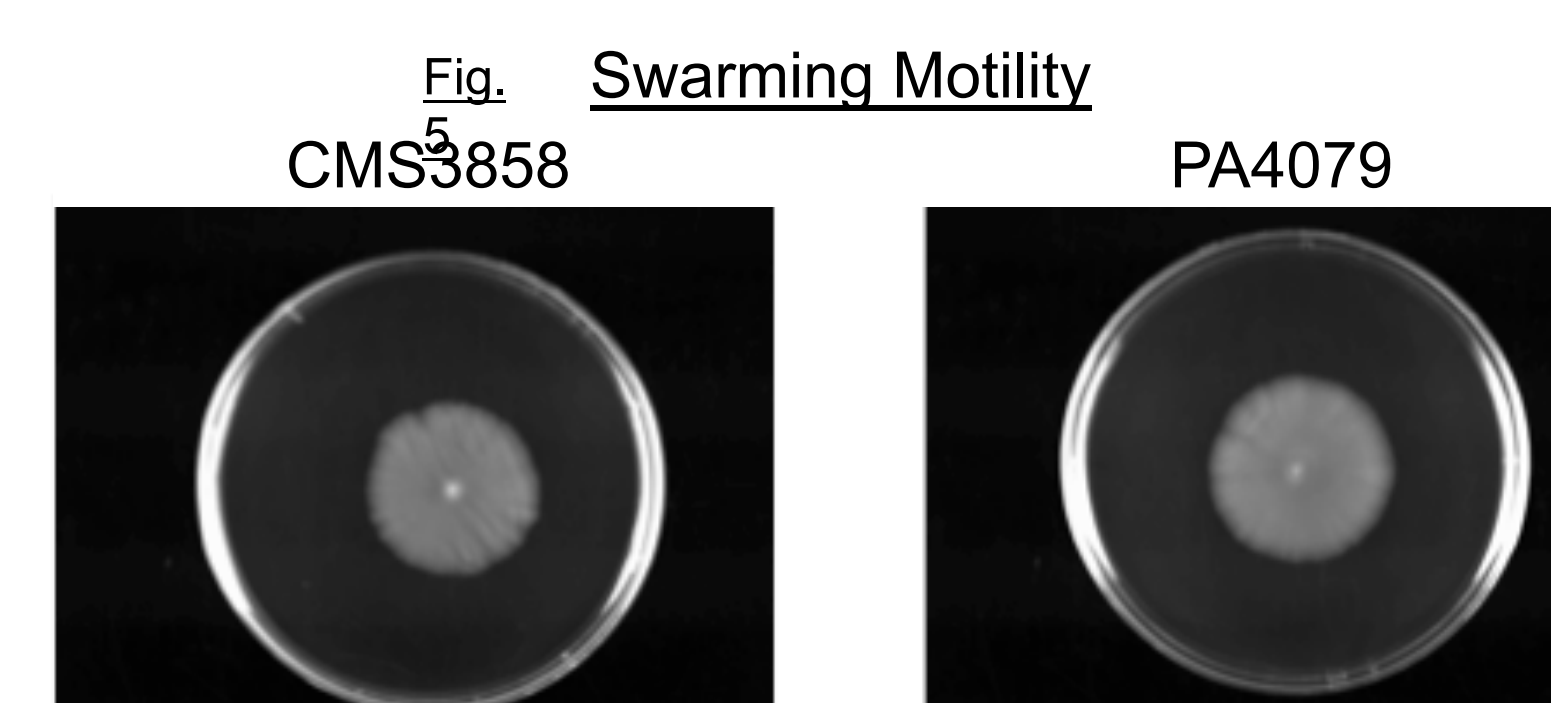
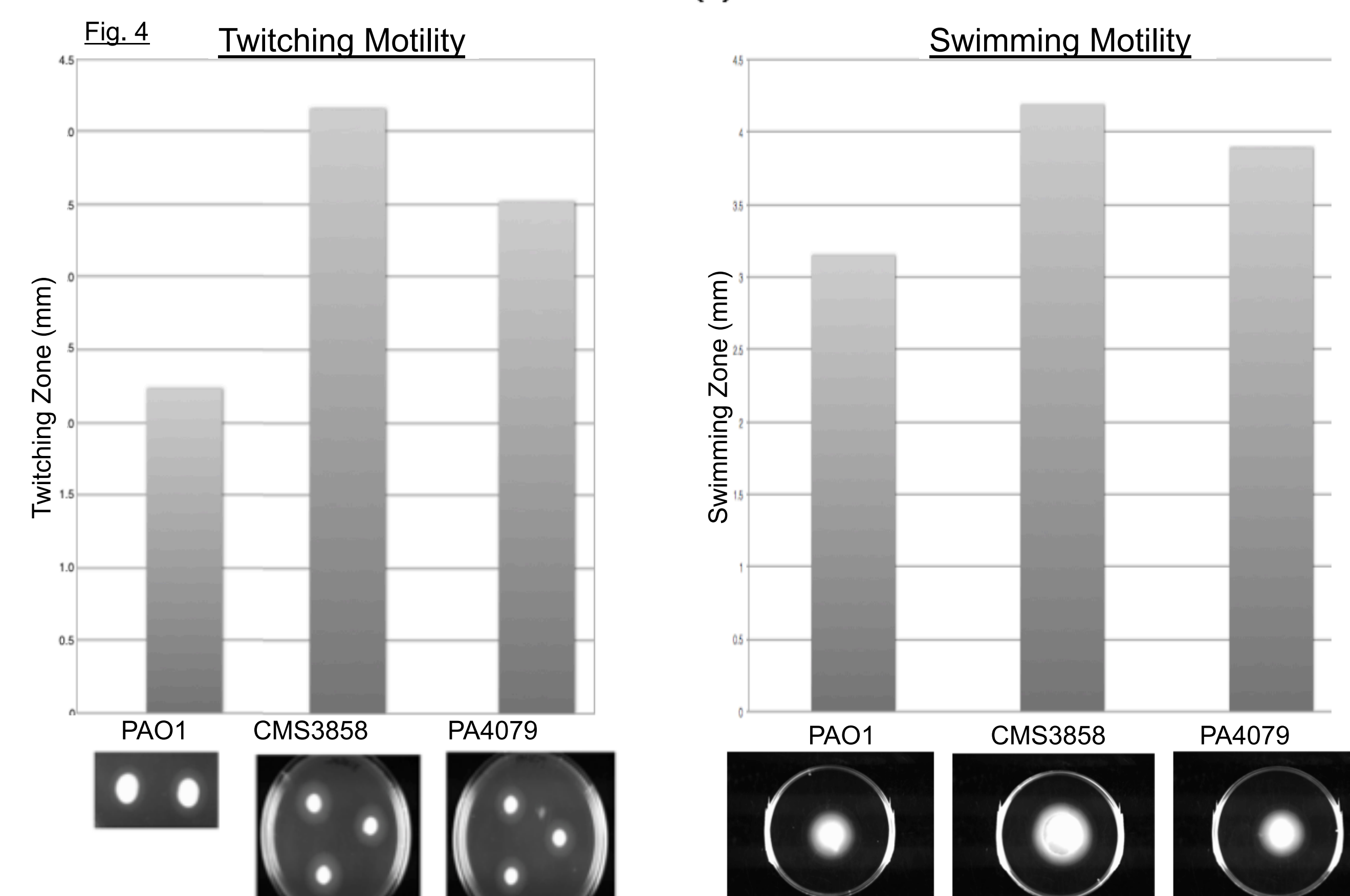
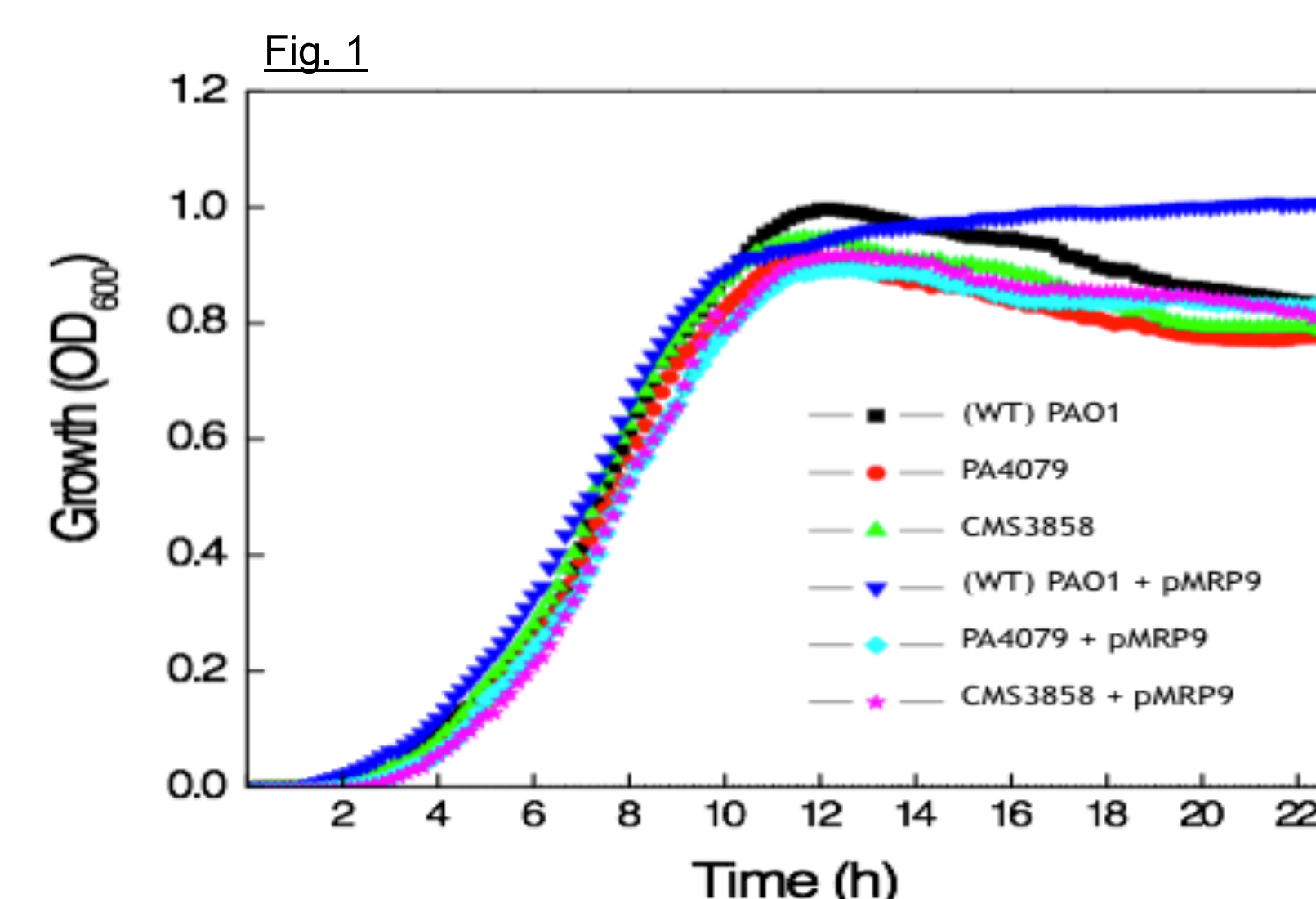
Bacterial biofilms are communities of bacteria which grow on various surfaces. They are composed of the bacteria themselves and an extracellular polymeric slime (EPS) matrix which they encase themselves within. Biofilms can be found in any environment where there is persistent water, such as oceans, pipes, and the human body. Biofilms are formed when free-floating, planktonic bacteria attach to a surface and grow. Bacteria in a biofilm use communication via cell signaling to coordinate the behavior of the whole biofilm population for a diverse array of functions, including expression of virulence factors, proliferation and dispersal. In cell signaling, diffusible chemicals are released by individual bacteria which can be "sensed" by others in the biofilm. A short-range cell signaling protein in *Myxococcus* was discovered to be responsible for social motility in biofilms. We have identified a single potential protein homolog in *Pseudomonas aeruginosa*, PA4079, for which we are testing to determine social motility differences between a wild-type strain of *P. aeruginosa* versus a strain with knockouts of the PA4079 gene. Understanding how cells within biofilms move and communicate could allow us to prevent infections and spread in the environments in which they thrive.

Methods

- Used two mutant strains of *Pseudomonas aeruginosa*: one with a transposon deletion (PA4079) and one with clean deletion of the gene (CMS3858), along with a wild-type control.
- All strains tagged with GFP for comparison; biofilms were stained with propidium iodide to compare live/dead cells before imaging.
- Utilized different media conditions (minimal v. rich) and temperature changes (37°C v. 22°C). Biofilms were rinsed daily with a sterile saline solution. Images were captured using an EVOS® imaging microscope to detect any phenotypic differences between the strains.
- Pyocyanin pigment production is controlled by the quorum sensing system, so we tested to see if the mutation influenced diffusible cell-to-cell signaling.
- Tested twitching, swimming, and swarming motility to determine differences between mutants and wild type.

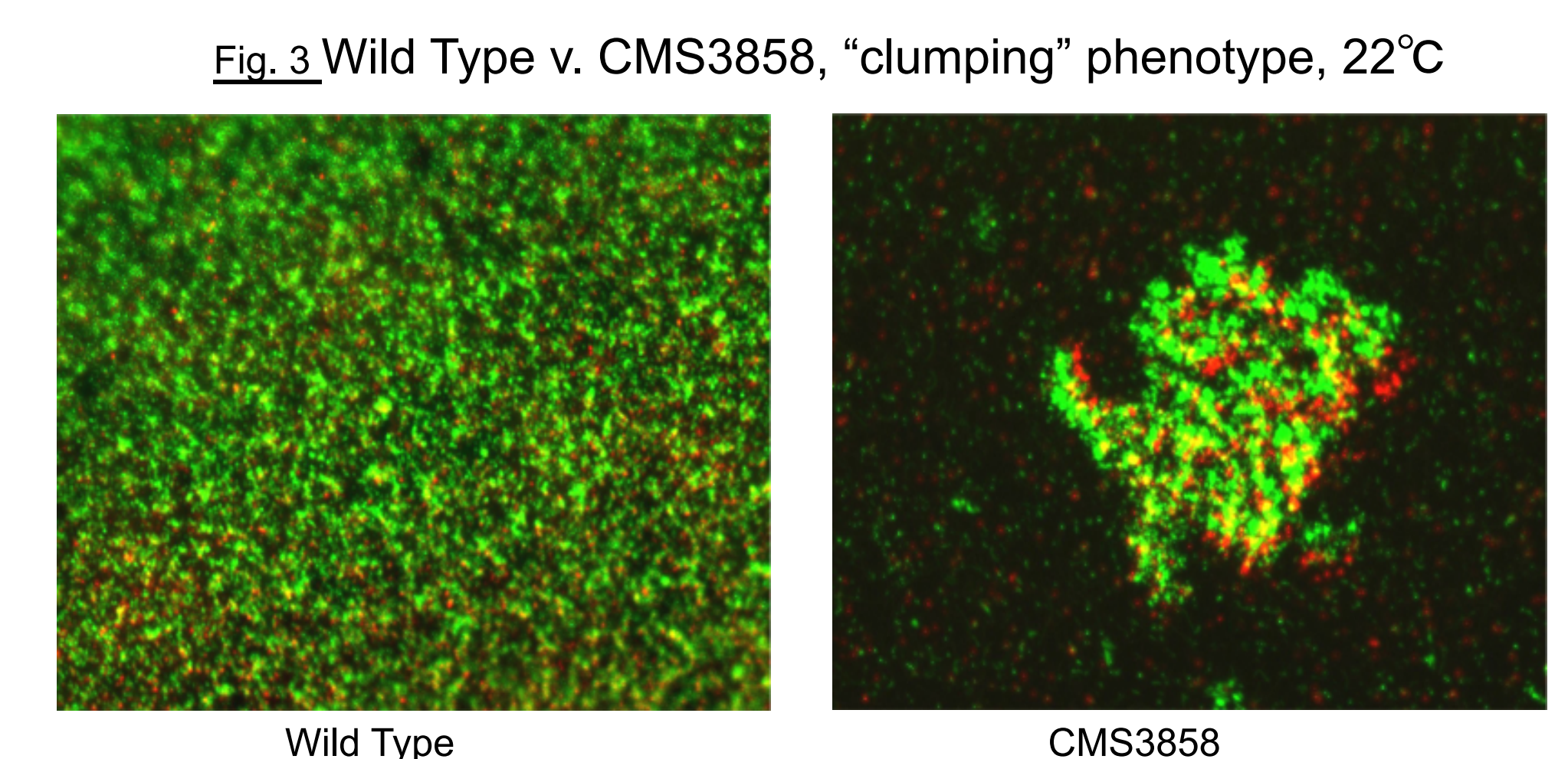
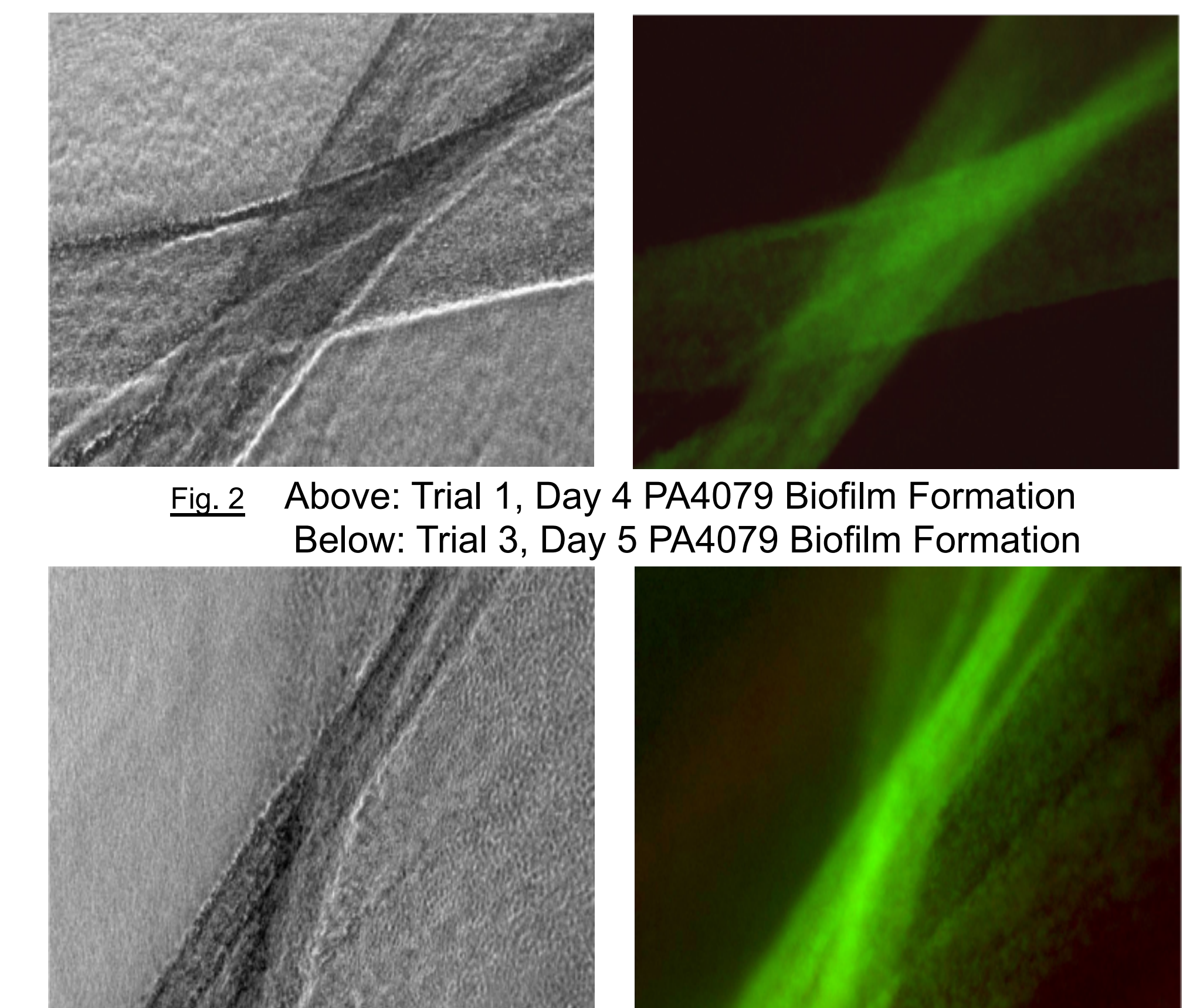
Results

- Growth rates were similar between both mutant strains and the wild type strain under minimal and rich media at 37°C (Fig. 1).
- Phenotype unique to the transposon-deletion strain when grown in rich media (Fig. 2).
- Minimal media at 22°C resulted in slower growth rates and biofilm progression in all strains when compared to rich media. Mutant strains formed "clumps" within the biofilm, where the wild type strain formed a consistent biofilm (Fig. 3).
- The mutation does not appear to influence pyocyanin.
- Mutants showed more twitching/swimming than wild type (Fig. 4).
- No observable difference in swarming motility (Fig. 5).



Conclusions

- Looking into time-lapse photography to help us understand how these biofilms form and thrive, as well as capture movement within the biofilms.
- Will also be manipulating different metabolic processes (i.e. starvation and restriction) to see if the potential protein homolog influences cells when grown under more stressful conditions.



Acknowledgements

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